*nptII* or *bar* genes and analysis regarding their inheritance in subsequent progeny. He mentioned how he could recover mature transgenic plants by *in vitro* grafting of transformed shoots onto 5-day-old-dark grown seedlings. B. K. Sarmah (AAU, Jorhat) reported the work carried out at CSIRO, Australia on introduction of protease inhibitor gene from *Nicotiana alata* and α-amylase inhibitor gene from common bean into Australian desi chickpea cultivars. The $T_1$ seeds subjected to insect bioassay showed resistance to *Callosobruchus maculatus*. He also presented the work initiated at Assam Agricultural University on regeneration and transformation using Indian chickpea cultivars. D. V. Amla (NBRI, Lucknow) reported efficient regeneration through direct shoot formation, organogenesis and somatic embryogenesis from different explants on different hormone combinations. The regenerated shoots could be rooted and established in soil with 20–28% survival rate. Optimization of several parameters like processing of explants, *Agrobacterium* concentration, co-culture conditions and selection regime resulted in transformation frequency of 0.02–0.5%. The expression of 2.2-kb truncated *Bt-cry1Ac* was also achieved in primary transformants. J. Sen (NCPGR, New Delhi) discussed the ongoing research on improvement of nutritional quality by the transfer of desensitized aspartate kinase gene coding for the first enzyme of asparate amino acid biosynthetic pathway leading to the synthesis of some essential amino acids and also on developing fungal resistance against *Ascochyta rabiei* by incorporating antifungal protein genes isolated from *Dahlia merckii* and *Heuchera sanguinea*. In both the studies, primary transformants recovered on selection medium were positive in PCR studies and further molecular analysis on integration and expression of transgenes was to be carried out. P. Anand Kumar (NRCPB, IARI, New Delhi) presented results concerning the *in vitro* regeneration and *Agrobacterium*-mediated transformation of another important grain legume, pigeon pea. His group had regenerated chimeric pigeon pea plants expressing the *Bt-Cry1Ac*.

The round-table discussion noted that:

(a) Success has been achieved in regeneration via direct or indirect organogenesis and somatic embryogenesis from diverse explants on different media of many genotypes. But rooting of shoots, establishment of rooted shoots to soil and conversion of embryos to plants still pose problems, in addition to the genotype dependency of the methods. Moreover, regeneration protocols based on somatic embryogenesis are lengthy and difficult to reproduce. Regeneration of plants from protoplasts and anther cultures has not been achieved. For rooting of shoots, *in vitro* grafting method was suggested as the grafted plants established at higher frequency and grew faster compared to direct rooted plants.

(b) The transgenic chickpea plants have been obtained by both particle bombardment as well as by *Agrobacterium*-mediated transformation techniques. However, most of the workers have employed direct shoot organogenesis from cotyledonary node explants for *Agrobacterium*-mediated transformation and used *uidA* as reporter and *nptII* as selectable marker genes under the control of nos or CaMV 35S promoters. The frequency of transformation has remained low and the inheritance and stability of gene expression have not been studied. It was suggested that the other reporter genes, i.e. *gfp*, *luc*, etc. and selectable markers and the tissue and developmental stage-specific promoters (identified and isolated from chickpea for higher expression of transgenes) should be tried.

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**Pawan K. Jaiwal* and Sonia**, Department of BioSciences, M.D. University, Rohtak 124 001, India; and **K. C. Upadhyaya**, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India and National Centre Plant Genome Research, JNU Campus, New Delhi 110 067, India
*For correspondence.

**RESEARCH NEWS**

# Molecular link between diabetes and obesity: The resistin story

*Nasreen Z. Ehtesham*

## Diabetes

Diabetes continues to be one of the oldest diseases widespread across geographical and genetic boundaries. Diabetes is defined as a condition that occurs because of lack of insulin or presence of factors opposing the action of insulin, resulting in an increase (hyperglycaemia) in blood glucose levels[1]. Poor control of diabetes leads to complications, which could damage small blood vessels throughout the body, thereby causing impaired delivery of nutrients and hormone to the tissues and eventually causing tissue-damage. Several tissues could be affected, causing corresponding diseases of the retina (retinopathy), renal glomerulus (nephropathy), nerve sheath (neuropathy) etc. Macrovascular complications have been associated with hypertension and dyslipidaemia, both of which are directly or indirectly linked to cardiovascular, cerebral, renal and peripheral atherosclerotic vascular diseases.

## Forms of diabetes

There are two major kinds of diabetes: type 1 diabetes, known as insulin-dependent

diabetes mellitus (IDDM), and type 2 diabetes known as non-insulin-dependent diabetes mellitus (NIDDM). In addition to these two common forms, which account for about 95% of the total diabetes cases reported worldwide, the WHO has recognized four additional types of diabetes. These include malnutrition-related diabetes mellitus (MRDM), gestational diabetes (GD), impaired glucose tolerance (IGT) and diabetes associated with other conditions and symptoms such as pancreatic disease, disease of hormonal aetiology, drug or chemical-induced or those as a result of abnormalities of insulin or its receptor or certain genetic syndromes.

IDDM, which results from the destruction of pancreatic cells through an autoimmune process, eventually leads to absolute insulin deficiency. There is a genetic predisposition, which is triggered by environmental and/or nutritional factors[2]. Type 2 diabetes or NIDDM accounts for 75% of all diabetes cases and is characterized by peripheral insulin resistance and relative insulin deficiency. Insulin resistance takes the form of a decrease in glucose storage or oxidation properties of skeletal muscles due to reduced activity of the enzymes glycogen synthase and pyruvate dehydrogenase, respectively[3]. While type 1 diabetes can be treated by daily injection of insulin, treatment of type 2 diabetes typically includes diet control, exercise and oral medication using hypoglycemic agents.

## Diabetes and obesity

Type 1 diabetes tends to increase around the time of puberty and declines thereafter. The incidence of type 2 diabetes increases with age and increasing obesity (particularly visceral or abdominal) and is more common in men than in women. The risk of developing NIDDM increases with a family history of diabetes or cardiovascular disease (particularly hypertension or dyslipidaemia) and lack of physical activity. Obesity, which is emerging as a major health problem in developed countries and is the second most common cause of preventable death in the USA[4,5], results from an imbalance between energy intake and energy expenditure[6]. It is characterized by a pathologic accumulation of triglycerides (fat molecules) in adipose tissues, thereby promoting insulin resistance in muscles, liver and other tissues. While studies of twins have clearly attributed the variation in body mass index to genetic factors, obesity is a clear reflection of an interaction of development and environment with genotype[7].

## Molecular basis of obesity

Despite the very strong linkage between obesity and type 2 diabetes (80% of NIDDM patients are obese), the molecular link has remained a mystery. Given the high levels of free fatty acid (FFA), products of triglyceride metabolism, in the blood stream in obese people compared to non-obese individuals, along with the ability of FFA to induce insulin resistance in peripheral tissues[8], these biomolecules were naturally suspected as a link between obesity and diabetes. Single gene mutations in proteins secreted by adipocytes such as leptin, TNF$\alpha$, adipsin etc. have been the target of studies associating obesity with genes. However, screening of thousands of obese subjects for leptin genes[9,10] or leptin receptor gene[11] have failed to generate an association of mutation in these genes with obesity. Mutations of genes regulating adipocyte differentiation or triglyceride storage have been associated with obesity in humans. Mutation in the nuclear receptor peroxisome proliferator activating receptor gamma (PPAR$\gamma$), which is an important regulator of adipocyte differentiation and modulator of intracellular insulin signalling events, has recently been found to predispose people to obesity[12,13] and hypertension[14]. The high expression of PPAR$\gamma$ in activated macrophages which play a role in atherosclerosis, provides support for the anti-atherogenic role of PPAR$\gamma$[15–17].

## Resistin: The novel adipocyte protein

Thiazolidenediones (TZDs), a class of antidiabetic drugs, enhance target tissue sensitivity to insulin by regulating target genes involved in insulin-mediated signalling pathways[18,19]. TZDs function as a high-affinity ligand for PPAR$\gamma$, however the gene target(s) for TZD-bound PPAR$\gamma$ that regulates insulin sensitivity has not been described. In a recent study[20,21], a novel adipocyte-specific gene was identified which was suppressed by TZD. This gene, called resistin (*resist in*sulin) was discovered while screening for genes that are induced during adipocyte differentiation, but down-regulated in mature adipocytes exposed to TZD. The level of resistin protein was high in adipocyte in a variety of rodent models of obesity – both genetic and diet-induced. Circulating resistin levels in mouse serum decreased with the administration of the antidiabetic drug, roziglitazone and other TZDs, including pioglitazone and troglitazone. Resistin-like molecules (RELMs) have been identified from different tissues in humans and rodents, which together with resistin form a class of tissue-specific signalling molecules[22].

The 592 nt resistin mRNA encoded a protein of 11 kDa which was processed to a secreted 9 kDa protein after the cleavage of a 20-amino acid signal peptide. The human homologue of resistin has been mapped to chromosome 19. Resistin mRNA level was found to be expressed as a function of white adipose deposit and sex of the animal, with the highest level seen in female's gonadal fat. While normal rat serum had clearly detectable immunoreactive levels of resistin, which decreased dramatically after a 48 h fast, and was reversed by refeeding. Mice fed on diet containing high fat for 8 weeks showed markedly elevated serum resistin levels. Genetically defined *ob*/*ob* (obese hyperglycaemia) and *db*/*db* (diabetic) mice had elevated resistin levels.

## Resistin, obesity and diabetes

To test the direct involvement of resistin in insulin resistance, both *in vitro* and *in vivo* mouse experiments were performed. 3T3 L1 adipose cells cultured *in vitro* showed a natural secretion of resistin into the culture medium. These cells, which are commonly used to study insulin stimulated glucose uptake, when treated with purified recombinant resistin protein showed in reduced insulin-stimulated glucose uptake. In complementary experiments, treatment of 3T3 L1 adipocytes with immunoglobulin $\gamma$ (IgG) purified from resistin antiserum increased insulin-stimulated glucose uptake by 42%, whereas control IgG had little or no effect.

Armed with these *in vitro* data suggesting the role of resistin in decreasing insulin-stimulated glucose uptake, more direct animal model experiments to link resistin to obesity and diabetes were performed. Purified recombinant resistin was admin-

istered intraperitonially (i.p.) to C57B1/6J mice and glucose tolerance was measured. Peak blood glucose level increased in the resistin-treated mice, compared to control-injected mice. This was followed by a concomitant increase in insulin levels. These results, though statistically not very significant ($P = 0.004$), nevertheless suggested an impairment in glucose tolerance *in vivo* as a direct function of resistin administration. Resistin neutralization experiments in mice were carried out to further document the involvement of resistin in insulin resistance. Diet-induced insulin-resistant obese mice when administered anti-resistin IgG therapy showed a significant decrease in blood glucose and reversibly reduced hyperglycaemia. Anti-resistin IgG-treated mice showed much improved insulin sensitivity compared to non-specific IgG treated mice. These experiments clearly pointed to the involvement of resistin in mediating insulin resistance in diet-induced obesity.

## Resistin: The unanswered questions

This latest report by Steppan *et al.*[22] while providing an important link between diabetes and obesity, additionally projects resistin as a candidate to explain the anti-diabetic effect of the TZD class of drugs. It also attempts to explain the mechanism by which excess adiposity leads to insulin resistance. Several issues however, remain to be addressed. What are the other physiological targets of resistin? Is there a receptor for resistin? What are the normal physiological functions of resistin? How is resistin level regulated by TZD? What is the mechanism(s)? Is there an expression-related association of resistin with predisposition to obesity and/or diabetes? What is the status of resistin in naturally obese human populations vis-à-vis those that are diet-induced? It would also be interesting to study transcriptional regulation of resistin and its association with obesity in the obese rat model developed by the National Institute of Nutrition, Hyderabad. Can resistin be used as a diagnostic marker for type 2 diabetes mellitus and other obesity-related complications? While these questions will be the focus of investigations in the coming years and will keep nutritionists, molecular and cellular biologists and pharmacologists occupied for the next several years, the resistin story is a rare example of an existing drug paving the way for the discovery of new drugs.


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*Nasreen Z. Ehtesham is in the National Institute of Nutrition, Hyderabad 500 007, India (e-mail: nasreen_e1@hotmail.com).*